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COMPARISON OF THE α-GLUCOSIDASES OF SACCHAROMYCES PRODUCED IN RESPONSE TO FIVE NON-ALLELIC MALTOSE GENES

HARLYN O. HALVORSON, SARA WINDERMAN AND JOHN GORMAN

Department of Bacteriology, University of Wisconsin, Madison, Wisc. (U.S.A.)

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SUMMARY

A comparison has been undertaken of the α-glucosidases produced in Saccharomyces in response to 5 non-allelic maltose genes. The partially purified enzymes were found to be indistinguishable in regard to heat inactivation, electrophoretic mobility, chromatography from CM-cellulose or DEAE-cellulose columns, neutralization with specific antiserum, or substrate specificity. In each of the genotypes only a single species of α-glucosidase was observed.

INTRODUCTION

Biochemical studies on the effects of mutations have led to the conclusion that not only do distinct structural genes control specific protein synthesis but also certain mutations in this region can give rise to structurally altered proteins. Multiple molecular forms of a given enzyme have been recognized not only within organisms of the same species[1] but also within a single organism[2]. In the latter case multiple forms of the same gene undoubtedly exist in a given organism.

Multiple gene control of a given enzyme species has been well documented in yeast. A well known example of this is the case of the polymeric genes for maltose. Analyses of interbreeding yeast strains have revealed at least six such unlinked genes. M_1 through M_3 were isolated from *Saccharomyces cerevisiae*[3], M_5 from *Saccharomyces diastaticus*[4], M_6 from *Saccharomyces carlsbergensis*, and M_4 from *Saccharomyces cerevisiae*[6] through induced, as well as spontaneous, mutation. Several unlinked maltose genes have been recognized in other species of yeasts[7-10].

Are identical gene products produced by the various M genes? M_1 and M_2 produce a rapid maltose fermentation and M_3 a slow maltose fermentation[3]. M_1 and M_4 were reported to ferment maltose and sucrose while M_2 and M_3 fermented only maltose[11]. Analyses of α-glucosidase extracts of maltose-grown yeasts have also suggested molecular heterogeneity. SAWAI[12] observed two maltose-splitting enzymes in *Candida tropicalis*, one active at pH 4.0 and the other at pH 6.8. TERUI *et al.*[8], investigating the α-glucosidases of *Saccharomyces cerevisiae* (Rasse II) observed two malrose-splitting fractions differing in heat stability and electrophoretic mobility. The present paper, following the isolation and characterization of the α-glucosidase

produced in response to M_1 in *Saccharomyces italicus*[13], is an attempt to characterize the proteins determined by the M_1 through M_4 and M_6 genes. The dosage effects of these same genes in regulating α-glucosidase synthesis are reported elsewhere[14].

METHODS

Strains employed and growth of cells

A variety of haploid, interbreeding yeast strains, known to contain different M genes, were used in this study. The strains employed, their source and genotypes are shown in Table I.

TABLE I

PURIFICATION OF α-GLUCOSIDASES FROM VARIOUS GENOTYPES

Strain	Source	Genotype	α-Glucosidase	
			Units/ml	Units/mg protein
H184	WINGE	M_1	155 000	115 000
56-10	WINGE	M_2	44 300	76 000
H108	WINGE	M_3	20 000	100 000
H131	WINGE	M_4	36 000	124 000
303-65	WINGE	M_6	28 800	88 000
1198-6D	HAWTHORNE	MA_2	56 500	47 500

For the preparation of enzyme extracts, the yeast cultures were grown in 15-l quantities in a maltose broth medium at 30° under vigorous aeration. The cells were collected by centrifugation, washed, frozen and stored at −20°.

Preparation of enzyme extracts

For the preparation of enzyme extracts, 50 g each of frozen cell paste were used. The cells were thawed, autolized and the enzyme partially purified (up to Fraction IV) as previously described by HALVORSON AND ELLIAS[13]. The initial purification steps in each case involved differential centrifugation of the extract, and absorption and elution of the α-glucosidase from calcium phosphate gel. Extracts from strain H84 and H131 were then placed on a DEAE-cellulose column and eluted with a linear gradient of NaCl (0.1 M). The peak tubes were combined and dialyzed against 0.01 M Tris–succinate buffer (pH 6.8). The enzyme from H108 was further purified by zone electrophoresis[13]. The similar fractions from strains 1198-6D and 303-65 were fractionated by the addition of solid ammonium sulfate. The precipitates obtained from the 50–75% saturation were retained, dissolved in 0.02 M Tris–cacodylate buffer (pH 6.8) and dialyzed against the same buffer. Table I summarizes the final specific activities of the various enzyme preparations. The purification of the enzymes from the crude extracts varied from 16-fold for MA_2 to 35-fold for the M_3. The final enzyme preparations were stored at −20° until used. There was little loss of enzyme activity during storage.

Immunization

Antiserum against the α-glucosidase from 1198-6D strain (Table I) was obtained by immunization of young adult rabbits. 1 mg of the enzyme preparation (previously dialyzed against saline) was injected intravenously and 10 days later each rabbit received nine further intravenous injections of about 2 mg of protein on alternate days. The total dose consisted of approximately 20 mg of protein per animal. 10 days after the last injection the rabbits were bled white and the serum separated. Normal rabbit serum was obtained from uninjected animals. The serum α-glucosidase in the antiserum was inactivated by heating the antiserum at 56° for 30 min.

Enzyme neutralization

Enzyme neutralization was measured essentially by antigen titrations. These were carried out at low concentrations of antigen (approx. 300-400 units of enzyme/ml) by mixing samples of enzyme solution (previously dialyzed against saline) with varying concentrations of antisera and saline in a total volume of 1.5 ml. A precipitate was rapidly formed after mixing the solution. The mixture was allowed to stand 15 h at 4° to aid in the precipitation. The suspensions were centrifuged and the supernatant retained for assay. In repeated experiments the error did not vary by more than 10%. In antigen excess the amount of enzyme activity neutralized was found to be directly proportional to the quantity of antibody added. Repeated estimations of the neutralization titre against a single enzyme preparation varied within 10–15%. The precipitate was inactive and washing of the precipitate did not lead to the appearance of active α-glucosidase.

Analytical methods

The α-glucosidase activity was estimated by following the continuous release of *p*-nitrophenol from *p*-nitrophenol-α-D-glucopyranoside[13].

Protein was routinely determined colorimetrically with Folin–Ciocalteu reagent according to the procedure of LOWRY *et al.*[16] using crystalline egg albumin as a standard.

Reagents

DEAE-selectocel, 0.9 mequiv/g, and CM-cellulose, 0.8 mequiv/g, were obtained from the Brown Company. Maltose and turanose were obtained from the California Foundation for Biochemical Research. Mercaptoethanol was obtained from the Mathison Company, Inc. Tris was obtained from the Sigma Chemical Co. Deionized glass-distilled water was used for all experiments.

RESULTS

If the enzymes produced in response to the different M genes of yeast are not of the same molecular species, then one might expect that this would be reflected in their specificities or physico-chemical properties. In order to more critically compare these enzymes, partially purified α-glucosidase preparations, either singly or in

mixture, were subjected to a variety of fractionation procedures designed to select for different properties of proteins. Although enzyme preparations which have been purified about 30-fold (Table I) were employed in experiments reported below, a number of parallel experiments was carried out with identical results. In crude extracts only a single enzyme active against *p*-nitrophenyl-α-D-glucopyranoside was recognized by heat inactivation, electrophoresis, column-chromatography and specific neutralization with antisera. In both electrophoresis and column-chromatography fractionation parallel assays were carried out with maltose as the substrate. The ratios of hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside: maltose were constant and identical to the purified α-glucosidase. In addition the antisera precipitation removed both *p*-nitrophenyl-α-D-glucopyranoside-and maltose-hydrolyzing activity in parallel, supporting the conclusion that only a single α-glucosidase species was present in the crude extracts.

Heat inactivation

The thermal inactivation of α-glucosidase from *S. italicus*[13] at 50° was first order. In *S. cerevisiae*, TERUI *et al.*[8] reported two maltose-splitting enzymes. After heating for 5 min at 52.5°, one was 83–91% inactivated while the other was only 64% inactivated. The kinetics of inactivation were not investigated.

Among the α-glucosidases produced in response to the M genes, preliminary studies showed that they were rapidly heat-inactivated above 50° in accordance with first-order kinetics. In order to compare more carefully the properties of the M_1 through M_4 and the M_8 α-glucosidase, the kinetics of heat-inactivation of a

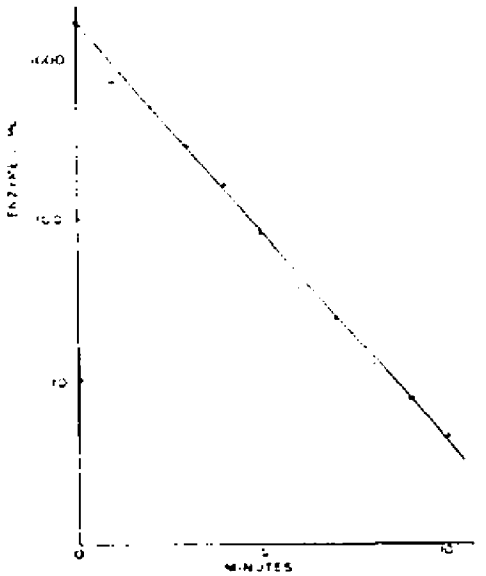

Fig. 1. Temperature inactivation of α-glucosidase mixture. To 1.8 ml 1/15 M phosphate buffer (pH 6.8) at 54.0° was added 0.2 ml mixture of enzyme (3400 units; 680 units each of α-glucosidase from M_1, M_2, M_3, M_4 and M_8). At intervals, 100 μl were removed to prechilled tubes containing 2.6 ml ice buffer and the enzyme assayed as previously described at 30°.

mixture of these enzymes was carried out at 54°. The results are shown in Fig. 1. The kinetics of heat-inactivation of α-glucosidase mixture are first order over a range of nearly 3 logs in activity. These findings are consistent with the involvement of a single enzyme species in this reaction.

Electrophoresis patterns of α-glucosidase

Our initial studies on the heterogeneity of enzymes in yeast capable of hydrolyzing α-glucosides was initiated more than 10 years ago in collaboration with Dr. S. SPIEGELMAN. In the early experiments it was observed that in crude extracts of *S. cerevisiae*, carbohydrases active against α-methyl-glucoside, sucrose, maltose and turanose could be separated into at least 3 components by starch zone-electrophoresis. Similar findings have been recently reported by TERUI *et al.*[8], and the α-methyl-glucoside-hydrolyzing enzyme identified as isomaltase.

In an attempt to further test the possibility that multiple types of α-glucosidase are produced in response to the various M genes, mixtures of equal units of M_1 through M_4 and M_6 α-glucosidase were subject to electrophoresis on a vertical starch column at pH 7.5. The electrophoresis patterns of protein and enzyme activity are shown in Fig. 2. As can be seen, the α-glucosidase has a slightly higher mobility

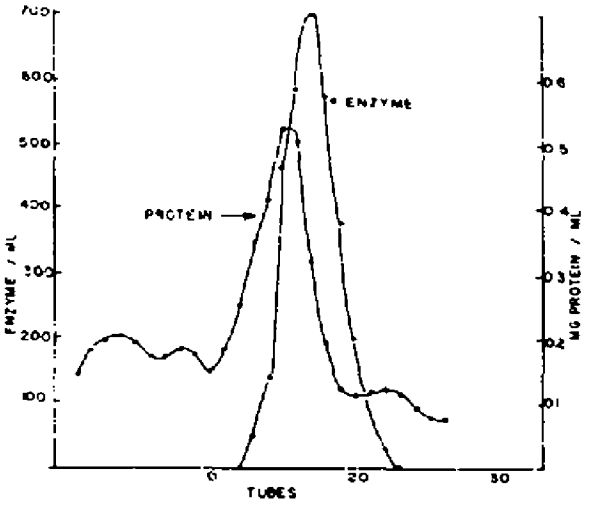

Fig. 2. Electrophoresis of a partially purified α-glucosidase. A vertical glass column (3 × 40 cm) was prepared containing washed potato starch. The column was equilibrated with 0.02 M Tris-thioglycolate buffer (pH 7.5). To the top of the column 2.0 ml of enzyme (7500 units; 1500 units each of M_1, M_2, M_3, M_4 and M_6) dialyzed against buffer was added. The enzyme was washed down the column with 90 ml of buffer. Vertical electrophoresis was carried out at 4°, 750 V and 10 mA for 13.5 h. The column was then eluted with buffer and the fractions (2.9 ml) analyzed for protein and enzyme. The total enzyme recovery was 104% (7800 units).

than the major peak of protein in the extract. Assays for the hydrolysis of maltose, α-phenolglucoside and turanose directly parallel the assay for *p*-nitrophenyl-α-D-glucopyranoside hydrolysis. There was no evidence of a second α-glucosidase observed by TERUI *et al.*[8]. The homogeneity of the enzyme peak suggests that a single electro-

phoretic species is involved. Similar results were obtained when the electrophoresis was conducted at pH 8.5 in pyrophospnate buffer.

In comparison the isomaltase has a slower mobility than the α-glucosidase[8,17]. Electrophoretic patterns of crude extracts of *S. cerevisiae* having the complementary genes for α-methyl-glucoside fermentation (MG_1, MG_2) show a single migrating species of isomaltase. When mixtures of α-glucosidase and isomaltase or extracts of strains containing both enzymes (*e.g.*, *S. cerevisiae* LK2G12) are subjected to electrophoresis the two enzymes can be readily separated.

Chromatography of enzyme mixtures

A mixture of α-glucosidases was dialyzed overnight against 10^{-3} M cacodylic acid, 0.02 M thioglycolate buffer (pH 6.8) and then subjected to chromatography on both CM-cellulose and DEAE-cellulose columns. The protein and enzyme profiles on these two columns are shown in Fig. 3. Both columns led to a fractionation of

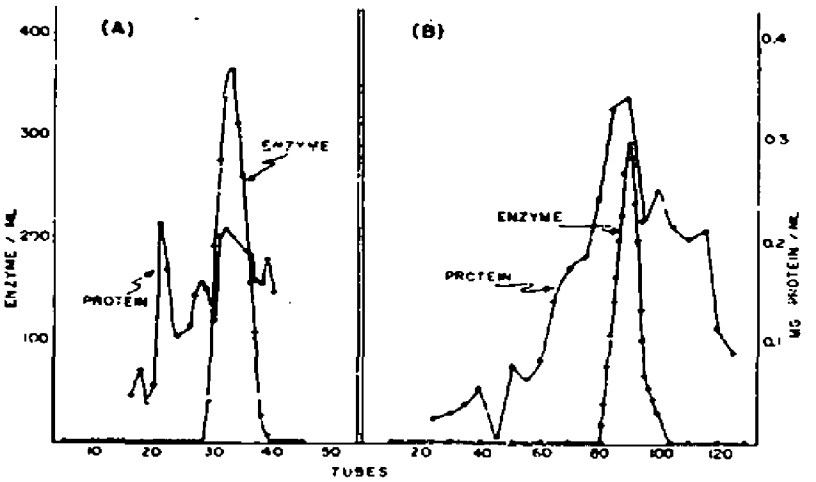

Fig. 3. Chromatography of a mixture of partially purified α-glycosidases. (A) CM-cellulose column. A glass column (27 × 1.7 cm) was prepared containing CM-cellulose. To this was applied 1.0 ml of enzyme (4000 units; 800 units each of M_1, M_2, M_3, M_4 and M_5). A NaCl gradient was applied by means of two reservoirs; one contained 500 ml of 10^{-2} M cacodylic acid and 0.02 M thioglycolate buffer (pH 6.8), and the other contained 500 ml of buffer containing 0.3 M NaCl. Fractions of 2.0 ml were collected at 4° and assayed for protein and α-glucosidase activity. The total enzyme recovery from the column was 95% (3800 units). (B) DEAE-cellulose column. The column and buffer were similar to that described above employing DEAE-cellulose type 40. To this was applied 1.5 ml of the enzyme mixture (6000 units). A NaCl gradient was applied from two reservoirs; 250 ml of buffer and 250 ml of buffer containing 0.5 M NaCl. 2-ml fractions were collected at 4° and assayed for protein and α-glucosidase activity. Total enzyme recovery was 94% (5600 units).

the remaining protein components of the preparations, although in both cases α-glucosidase eluted as a single symmetrical component. On both columns the position of the enzyme peak was the same as in elution patterns using individual enzyme preparations (Table I). When crude extracts were applied either to CM-cellulose or to DEAE-cellulose columns, only a single peak capable of hydrolyzing *p*-nitrophenyl-α-D-glucopyranoside was observed and this peak was superimposed on that obtained

with the partially purified enzyme preparations. The recovery of enzyme from both the CM-cellulose and DEAE-cellulose column was essentially quantitative.

Enzyme neutralization

The antiserum from normal as well as immunized rabbits contained a low level of serum maltase which hydrolyzed the substrate *p*-nitrophenyl-α-D-glucopyranoside.

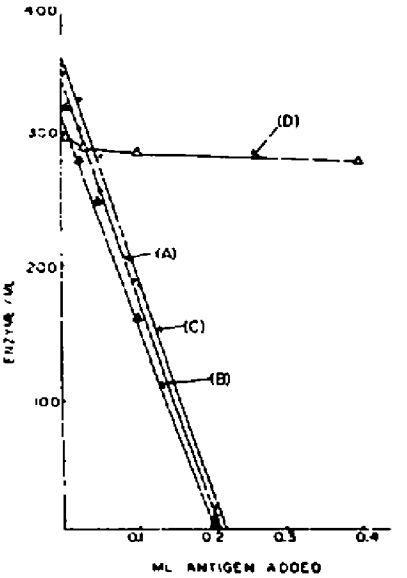

Fig. 4. Neutralization curve of α-glucosidase activity by anti-M_6 α-glucosidase antibody. Varying quantities of antiserum were mixed with (A) 345 units of M_2 α-glucosidase, (B) 320 units of M_6 α-glucosidase or (C) 360 units of a mixture containing 72 units each of M_1 through M_4 and M_6 α-glucosidase; (D) is a control containing normal serum and 300 units of M_4 α-glucosidase. After 15 h incubation at 4° the precipitate was removed and the supernatant assayed for α-glucosidase activity.

It was found that heating the antiserum preparation at 56° for 30 min destroyed the serum maltase without reducing appreciably the titre of anti-M_6 α-glucosidase. In the subsequent experiments heat-treated antiserum was employed.

Fig. 4 represents typical neutralization curves obtained when increasing amounts of anti-M_6 antibody are added to given quantities of α-glucosidase. It is clear that the neutralization of α-glucosidase proceeds in a strictly linear rate until over 97% or more of the enzyme is inactivated. The activities tested, either directly with the antigen–antibody solution or in the supernatant obtained after centrifugation of the antigen–antibody mixture, were the same. Furthermore, direct analysis of the precipitate or washed precipitate failed to detect the presence of enzyme. Increases in the concentration of substrate 5-fold above the saturation level did not reverse the inhibition caused by combination of the enzyme with antibody.

The slope of the inactivation is linear and follows the same rate for both the M_2, M_4 and a mixture of M_1 through M_4 + M_6 α-glucosidases. The amount of enzyme

activity neutralized is thus proportional to the quantity of antibody added and the neutralization titre of the antibody preparation is the same for the various α-glucosidases tested. It was further observed that the antibody had the same neutralizing activity against highly purified enzyme preparations as against enzyme in crude cell extracts.

The antigen–antibody reaction appears specific. When normal serum was employed there was only a slight decrease in the α-glucosidase activity. Antiserum against the M_6 α-glucosidase failed to neutralize or precipitate yeast isomaltase[18].

Partial absorption of antibodies

When α-glucosidase was added to the antibody preparation at levels at which α-glucosidase began to appear in the supernatant fluid, it was observed that all, or nearly all, the neutralizing antibodies to all five types of α-glucosidases were removed. When larger amounts of antiserum were employed, the excess antibody appeared in the supernatant. Table II shows the effect of anti-M_6 serum following

TABLE II

REMOVAL OF NEUTRALIZING ANTIBODIES FROM ANTI-α-GLUCOSIDASE IMMUNE SERUM BY ABSORPTION WITH α-GLUCOSIDASE

The antibody and enzyme solutions were mixed in a final volume of 1.5 ml saline and incubated for 15 h at 4°, after which the precipitate was centrifuged down and the supernatant fluid assayed for α-glucosidase activity and α-glucosidase-neutralizing ability.

Amount of anti-α-glucosidase serum (ml)	*Absorbing M_6 α-glucosidase (units)*	*Enzyme activity remaining in supernatant after absorption (%)*	*Neutralizing antibody titre (%) remaining (after absorption) against*	
0.02	200	92	M_6	2.0
0.10	200	4	M_6	0
0.20	200	0.8	M_6	44
			M_4	45
			M_1–M_6, MA_2 mixture	50

absorption by M_6 α-glucosidase. At the equivalence point, 200 units of α-glucosidase and 0.10 ml of anti-M_6 serum, there was a loss of both enzyme and antiserum. When twice the level of antiserum was used, the supernatant fluid still retained approximately half of the neutralizing titre against M_6, M_4 and a mixture of M_1 through M_4 + M_6 α-glucosidase. These findings as well as the results in Fig. 4 show that the neutralization equivalents determined from the neutralization slope and the absorption tests are similar and argue that the various enzymes are identical.

The α-glucosidases produced in response to M_1 through M_4, M_6 as well as an unmapped MA_2, are completely precipitated by excess antiserum against M_6 α-glucosidase. Normal serum, on the other hand, has little effect on the enzyme activity. These results are shown in Table III.

TABLE III

NEUTRALIZATION OF VARIOUS α-GLUCOSIDASES BY ANTI-M_4 α-GLUCOSIDASE ANTIBODY

Varying quantities of α-glucosidase were mixed with 0.3 ml of normal or anti-M_4 α-glucosidase, undiluted γ-globulin solution in physiological saline in a final volume of 1.0 ml and incubated for 14 h at 4°. The precipitate was centrifuged off and the enzyme activity remaining in the supernatant fluid was assayed.

Precipitating α-glucosidase		*Enzyme activity remaining in supernatant after incubation with*	
Type	*Quantity used (units)*	*Normal serum*	*anti-M_4 serum*
M_1	350	383	8
M_2	310	332	4.2
M_3	400	450	2
M_4	400	410	4
M_6	400	395	5
MA_2	330	336	0
MZ	460	470	1.5

Enzyme substrates and complexants

When *p*-nitrophenyl-α-D-glucopyranoside was used as enzyme substrate, a typical Michaelis–Menten relationship was observed for all the α-glucocidases tested. In the presence of complexants of α-glucosidases, competitive substrate inhibition of *p*-nitrophenyl-α-glocupyranoside hydrolysis was observed similar to that described for the α-glucosidase from *Saccharomyces italicus*[18].

In order to compare the specificity of the various enzymes, complexants were

TABLE IV

COMPLEXING PROPERTIES OF GLUCOSIDES FOR VARIOUS α-GLUCOSIDASES

The reaction mixture (3.0 ml) contained 0.06 M phosphate buffer (pH 6.8), 70 units of enzyme and various concentrations of substrate (*p*-nitrophenyl-α-D-glucopyranoside) with or without inhibitor. The final inhibitor concentrations employed were 0.1 M for maltose, α-methylglucoside and sucrose, and $3.33 \cdot 10^{-2}$ M for turanose and α-phenylglucoside. The affinities of these inhibitors for the enzyme were calculated from the equation:

$$\frac{1}{V_i} = \frac{1}{V_{max}}\left(K_m + \frac{K_m I}{K_i}\right)\frac{1}{S} + \frac{1}{V_{max}}$$

where I is the concentration of the complexant, S the substrate concentration, K_i the inhibitor dissociation constant, K_m the equilibrium constant for substrate and V_i the initial velocity (zero-order kinetics) in the presence of the complexants. PNPG, *p*-nitrophenyl-α-D-glucopyranoside.

Source of enzyme	*PNPG K_m ($M \cdot 10^4$)*	*Maltose K_i ($M \cdot 10^4$)*	*Sucrose K_i ($M \cdot 10^4$)*	*Turanose K_i ($M \cdot 10^4$)*	*α-Phenyl-glucoside K_i ($M \cdot 10^4$)*
M_1	2.0	317	370	114	5.3
M_2	2.0	320	303	107	3.8
M_3	2.1	358	225	78	3.1
M_4	1.9	354	471	100	4.0
M_6	1.9	330	350	150	–
MA_2	1.7	367	310	117	5.0

chosen which had previously been shown with the M_1 α-glucosidase to vary over at least two orders of magnitude. The complexing properties of these inhibitors, as well as the K_m for *p*-nitrophenyl-α-D-glucopyranoside hydrolysis, are shown in Table IV. The K_m for *p*-nitrophenyl-α-D-glucopyranoside hydrolysis was essentially the same, varying from 1.7 to $2.1 \cdot 10^{-4}$ M. Maltose, sucrose, turanose and α-phenolglucoside were all found to be competitive inhibitors and to show close agreement with each other for the various enzymes within the limit of determination of their affinity constants (K_i). These results are essentially the same as those observed previously for M_1 α-glucosidase.

All five enzyme preparations were similar in other respects. They all hydrolyzed sucrose, α-phenolglucoside, maltose and turanose in decreasing order. In addition, these enzyme preparations had similar pH optimums of about 6.8 and were inhibited by sulfhydryl agents and by amines.

The substrate specificity and genetic properties of the five α-glucosidases are, therefore, indistinguishable from the α-glucosidase previously purified and characterized in *Saccharomyces italicus*[13].

DISCUSSION

Comparison of the α-glucosidases

The existence of a variety of related enzymes involved in maltose metabolism in yeast has been suggested from differences in the specificity of disaccharide fermentation in various defined genotypes and from studies on cell-free extracts. Information on the specificity of α-glucosidase based on experiments with intact yeast cells may be misleading since the transport of α-glucosides is regulated by specific permeation system(s)[8,15,19,20]. This system(s) undoubtedly provides an explanation for the failure of selected yeast to ferment sugars (*e.g.*, sucrose[11,21]) in spite of the presence of intracellular enzymes capable of metabolizing these sugars. Additional control genes may also regulate the utilization of sugars in yeast. OSHIMA[22] observed a complementary gene for maltose fermentation in *S. cerevisiae* and ROBERTS *et al.*[9] reported an assimilatory gene and two polymeric, non-complementary, control genes for maltose utilization in *Saccharomyces oviformin*.

Multiple α-glucosidases have been implied from analysis of the hydrolytic activities of cell extracts[8,28]. In few of these studies have the strains of defined geneotypes been employed or have the enzymes been purified and characterized, making it difficult to decide if, in fact, the same class of enzymes are being measured. For example, SAWAI[12] observed two maltose-splitting enzymes in *Candida tropicalis* differing in their pH optimum. The acid maltose-splitting enzyme was subsequently characterized as an intracelluiar amylase with a broad specificity[24].

In the present experiments an analysis of the enzymes produced in response to the genes M_1 through M_4 and M_6 have been examined. The presence of any one of the M genes leads to the production of detectable levels of enzyme in uninduced cells and elevated levels in induced cells. It has not been possible to detect functional or structural differences between the five α-glucosidases. The methods employed were designed to detect differences in substrate specificities, size and charge, adsorption, heat stability and serological specificity. These findings are strongly

suggestive, but not conclusive, that proteins having identical amino acid sequences and tertiary structure are involved.

The phenotypes of the various M genes are also indistinguishable. In the recessive state there is no detectable enzyme in cell extracts whereas in the dominant state basal enzyme can be detected and the cells can be induced to produce higher levels of α-glucosidase. There are at least two explanations for these identical phenotypes of the 5 M genes: these genes represent a multiplicity of either regulatory or structural genes. In either case a single gene would be sufficient to allow α-glucosidase synthesis and maltose fermentation. In recent years two types of regulatory genes have been described[25]: an operator gene, which controls the function of an adjacent group of genes (operon) on the same chromosome and a regulatory gene which functions either by controlling inducer permeability or by the production of repressors or inducers. Since if the 5 M genes were operator genes one would have to postulate 5 unlinked structural genes, this possibility is analogous to the assumption that 5 structural genes exist. On the other hand, the assumption that these genes represent regulatory genes deserves serious consideration. The fact that in the dominant state the system is inducible suggests that an inducer-reversed repressor may well exist[14]. This is further supported by the observation that in heterozygous diploids inducibility is dominant.

Regulatory and structural genes differ in a number of respects. The former leads to quantitative changes in enzyme, whereas the latter may give rise to qualitative differences. In the case of the M genes, the available evidence which supports the latter hypothesis can be summarized as follows. In yeast recessive for the M genes there is no detectable α-glucosidase activity in spite of the fact that the methods employed should have detected a few enzyme molecules per cell. In non-induced strains carrying one or more dominant M genes, appreciable levels of enzyme can be readily detected. If the M genes were regulatory rather than structural genes they must lead to an all or none response of a structural gene to produce low levels of enzyme. In addition the inducibility of the system would require the postulation of yet additional regulatory genes. In all control systems carefully examined thus far induction or depression leads to a quantitative increase in enzyme syntheses whereas mutations or deletions in structural genes (or linked operator genes) can qualitatively control the presence or absence of the enzyme. The conclusion that these genes are probably structural genes is supported by gene-dosage experiments[14]. In diploids homozygous and heterozygous for the various M genes, the induced level of α-glucosidase increases arithmetically with additional dosage of the M genes. Such proportionality, while predicted for the behavior of structural genes, are difficult to reconcile with regulatory genes.

The existence of a multiplicity of identical genes in a single organism is a unique situation. The phenomenon in yeast may not be restricted to the α-glucosidases. Multiple gene control of sugar fermentation has been observed as well for the fermentation of α-methyl-glucoside[8,24], raffinose and sucrose[27] and may be a common situation in yeast. In these cases, however, the respective enzymes have not been isolated and compared. Since yeasts are primarily fermenters of sugars and disaccharides, one can readily imagine the selective advantage of the accumulation of additional genes involved in the production of carbohydrases. In fact, RUDERT AND HALVORSON[14] have demonstrated a strictly additive increase in α-glucosidase levels with increased dosages of M genes.

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REFERENCES

1 V. M. INGRAM, *Nature*, 180 (1957) 326.
2 C. L. MARKERT AND F. MØLLER, *Proc. Natl. Acad. Sci. U. S.*, 45 (1959) 753.
3 Ø. WINGE AND C. ROBERTS, *Compt. Rend. Trav. Lab. Carlsberg, Sér. Physiol.*, 24 (1948) 263.
4 R. B. GILLILAND, *Nature*, 173 (1954) 499.
5 Ø. WINGE AND C. ROBERTS, *Compt. Rend. Trav. Lab. Carlsberg, Sér. Physiol.*, 25 (1955) 331.
6 Ø. WINGE AND C. ROBERTS, *Compt. Rend. Trav. Lab. Carlsberg, Sér. Physiol.*, 25 (1950) 35.
7 C. C. LINDEGREN AND G. LINGEGREN, *J. Gen. Microbiol.*, 15 (1950) 19.
8 G. TERUI, H. OKADA AND Y. OSHIMA, *Technol. Rept. Osaka Univ.*, 9 (1959) 237.
9 C. ROBERTS, Ø. WINGE AND J. WYNANTS, *Compt. Rend. Trav. Lab. Carlsberg, Sér. Physiol.*, 32 (1961) 305.
10 D. HAWTHORNE, unpublished results.
11 Ø. WINGE, *Heredity*, 6 (1952) 263.
12 T. SAWAI, *Botan. Mag. (Tokyo)*, 69 (1956) 103.
13 H. HALVORSON AND L. ELLIAS, *Biochim. Biophys. Acta*, 30 (1958) 28.
14 F. RUDERT AND H. O. HALVORSON, in preparation.
15 J. J. ROBERTSON AND H. O. HALVORSON, *J. Bacteriol.*, 73 (1957) 186.
16 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
17 H. O. HALVORSON AND S. SPIEGELMAN, unpublished results.
18 J. GORMAN, unpublished results.
19 G. HARRIS AND C. C. THOMPSON, *Biochim. Biophys. Acta*, 32 (1961) 176.
20 A. SOLS AND G. DE LA FUENTE, in A. KLEINZELLER AND A. KOTYK, *Membrane Transport and Metabolism*, Pub. Czech. Acad. Sci., Prague, 1961, p. 301.
21 S. HESTRIN AND C. C. LINDEGREN, *Arch. Biochem. Biophys.*, 29 (1950) 315.
22 Y. OSHIMA, *J. Ferment. Technol.*, 38 (1960) 521.
23 GAD AVIGAD, *Bull. Res. Council Israel, Sect. A*, 7 (1958) 112.
24 T. SAWAI, *J. Biochem. (Tokyo)*, 48 (1960) 382.
25 F. JACOB AND J. MONOD, *J. Mol. Biol.*, 3 (1961) 318.
26 D. C. HAWTHORNE, *Heredity*, 12 (1958) 273.
27 Ø. WINGE AND C. ROBERTS, *Compt. Rend. Trav. Lab. Carlsberg, Sér. Physiol.*, 25 (1952) 141.